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Bt transgenes minimally influence maize grain yield and lodging across plant populations

Mitchell J Novacek[1], **Stephen C Mason**[2*], **Tomie D Galusha**[2], **Muhammad Yaseen**[3]

[1]DuPont Pioneer, York-West Research Center, 619 East 25th Street, York, NE 68467, USA
[2]Department of Agronomy and Horticulture, 279 Plant Science, University of Nebraska, Lincoln, NE 68583-0915, USA
[3]Department of Mathematics and Statistics, University of Agriculture, Faisalabad, Pakistan
*Corresponding author: E-mail: smason1@unl.edu

Abstract

Adoption of maize (*Zea mays* L) hybrids containing Bt (*Bacillus thuringiensis*) transgenes and increased plant population is widespread but little scientific research exists on their interactions with production environments in the Western Maize Belt of the United States. Two pairs of near-isogenic Bt and non-Bt maize hybrids were grown under rainfed and irrigated conditions from 2008 to 2010 at target populations from 49,300 to 111,100 plants ha^{-1} near Mead, NE. The objective was to determine the influence of the presence/absence of Bt transgenes for European corn borer [*Ostrinia nubilalis* (Hübner) - ECB] and corn rootworm [*Diabrotica* spp. - CRW] on maize yield and lodging across a range of target populations. Bt maize hybrids produced 0.6 Mg ha^{-1} more grain, 0.2 more ears m^{-2}, and 1.3 g heavier 100-kernel weight than non-Bt hybrids in absence of visible ECB and CRW rootworm pressure. Yield of Bt and non-Bt hybrids responded similarly to increasing target population, with Dekalb DKC 58-16 and 58-19 increasing from 11.0 to 13.1 Mg ha^{-1} and Dekalb DKC 61-69 and 61-72 from 11.8 to 12.7 Mg ha^{-1} as the target population increased from 49,300 to 111,100 plants ha^{-1}. Lodging increased linearly with increasing target population, with a greater increase in rainfed than irrigated environments. Lodging of Bt and non-Bt maize hybrids was inconsistent. Grain yield, seed and insecticide cost, likelihood of ECB and CRW infestation, and environmental concerns related to soil insecticide use should be the drivers when determining if Bt maize hybrid use is justified. If CRW resistance occurs, planting non-Bt maize hybrids along with application of soil insecticide is a viable alternative.

Keywords: Bt transgene, yield components, bulk density, lodging

Introduction

Matching of the best maize hybrids with optimum plant population and production environment is required to maximize grain yield. Maize plant population has continually increased due to increased «crowding stress» tolerance in modern hybrids and the resultant grain yield increase (Duvick and Cassman, 1999; Hammer et al, 2009). Development and adoption of transgenic Bt (*Bacillus thuringiensis*) traits has improved modern maize hybrids by limiting damage from European corn borer [*Ostrinia nubilalis* (Hübner) - ECB] and corn rootworm (*Diabrotica* spp. - CRW), thereby protecting grain yield (Vaughn et al, 2005). This has increased harvestable maize grain yield (Stanger and Lauer, 2006), reduced use of chemical insecticides, and improved grain quality. Transgenic maize hybrids occupy 90% of maize area in the United States (USDA-ERS, 2013). Recently, CRW has overcome certain Bt transgenes (Gassmann et al, 2011) renewing the need for evaluation of crop rotation, non-Bt hybrids, and soil insecticide application. Little scientific research exists on interactions of these practices with environments in the Western Maize Belt of the United States.

Maize grain yield is related to the effects that increasing plant population has on plant morphology and physiology and grain yield components. Increased plant population leads to a greater leaf area index (LAI) at silking (Cox, 1996), which increases interception of photosynthetically active solar radiation (Tollenaar and Aguilera, 1992). However, per plant biomass at high plant population is reduced (Maddonni and Otegui, 2004). This decrease in per plant biomass causes a decrease in photosynthetic rate per plant and can increase plant barrenness at high plant population (Edmeades and Daynard, 1979; Maddonni and Otegui, 2004). The economic optimum plant population for Bt and non-Bt hybrids has been found to be the same in Wisconsin (Stanger and Lauer, 2006) and Illinois and Iowa (Coulter et al, 2010). Genetic improvements have resulted in an increase in number of ears per plant and kernel weight and reduced root and stalk lodging in modern maize hybrids (Duvick, 2005).

Increasing plant population alters yield components by decreasing the number of ears plant^{-1} (Tollenaar et al, 1992; Otegui, 1995), kernels ear^{-1} (Westgate et al, 1997; Maddonni and Otegui, 2006), and kernel weight (Otegui, 1995; Westgate et al, 1997; Maddonni and Otegui, 2006). Kernel weight is more stable than other yield components as plant population increases (Begna et al, 1997; Westgate et al,

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1997; Maddonni and Otegui, 2006). Kernel weight is influenced by source-sink relationships during grain fill (Borrás and Otegui, 2001; Gambín et al, 2006), with increased kernel weight occurring as irradiance and grain-fill duration increases.

Stalk lodging (plant breakage) and root lodging (plants fallen over) affect maize grain yield and harvestability (Sibale et al, 1992). Increasing plant population results in increased lodging potential due to increased plant and ear height and stalks with reduced diameters (Stanger and Lauer, 2007; Novacek et al, 2013), decreased rind thickness (Stanger and Lauer, 2007), and premature death of pith tissues along with increased rates of stalk rots (Dodd, 1977). Increased lodging often nullifies the grain yield increase from increased plant population (Olson and Sander, 1988). Bt maize hybrids have been reported to lodge less than non-Bt hybrids; however, results are inconsistent (Stanger and Lauer, 2007).

The objective of this research was to determine the influence of the presence/absence of Bt transgenes for ECB and CRW on maize grain yield and lodging across a range of plant populations in rainfed and irrigated environments in East-Central Nebraska.

Materials and Methods

Field experiments were conducted in rainfed and center-pivot irrigated environments at the University of Nebraska Agricultural Research and Development Center near Mead, NE (41°9'N, 96°27'W) in 2008, 2009, and 2010. Filbert silt loam (fine, smectitic, mesic Vertic Argialboll) with 0 to 1% slopes (USDA-NRCS, 2011) was the predominant soil type for the 2008, and 2009 irrigated environments and the 2010 rainfed environment. The predominant soil type for the 2008 and 2009 rainfed environments was Yutan silty clay loam (fine, silty, mixed, superactive, mesic Mollic Hapludalf) with 2 to 6% slopes. Maize was the previous crop in all environments. Soil characteristics included pH of 5.5 to 6.0, organic matter concentration of 33 to 38 g kg^{-1}, and K concentration of 250 to 340 ppm. The P concentration varied and recommended rates of P_2O_5 were applied and incorporated before planting based upon soil test results (Shapiro et al, 2008). Nitrogen applications were made based upon soil NO_3-N concentrations and an expected rainfed maize grain yield of 10.0 Mg ha^{-1} and an irrigated maize grain yield of 15.7 Mg ha^{-1} using University of Nebraska recommendations. In rainfed environments, 140 kg N ha^{-1} as anhydrous ammonia was applied on 9 Apr 2008, 25 Nov 2008, and 15 Apr 2010, while irrigated environments received 224 kg N ha-1 as anhydrous ammonia on 7 April 2008 and 26 Mar 2009. On 25 June 2009, an additional 84 kg N ha^{-1} as urea was surface broadcast on the irrigated environment to correct a visual N deficiency which was likely due to leaching/denitrification losses resulting from excessive early season rainfall.

A randomized complete block designed experiment with a split-plot treatment arrangement and three replications was used for each environment. Environments were considered to be year/water regime combinations. Main plots were target plant populations of 49,300, 61,700, 74,000, 86,400, 98,800, and 111,100 plants ha^{-1}. Plots were planted at rates above the target populations and thinned to the desired population at the V4 to V6 growth stages (Abendroth et al, 2011). Split-plots consisted of two pairs of near-isogenic hybrids: Dekalb DKC 58-16 and Dekalb DKC 58-19 (108-day relative maturity) and Dekalb DKC 61-69 and Dekalb DKC 61-72 (111-day relative maturity). All hybrids were glyphosate-resistant; additionally, hybrids Dekalb DKC 58-16 and 61-69 had the Bt transgenes for resistance to ECB and CRW. Plots were six 76-cm rows (4.6 m wide) by 9.1 m long.

A John Deere 7100 MaxEmerge mechanical maize finger pickup unit planter (Deere & Company, One John Deere Place, Moline, IL 61265-8098) with row cleaners in front of the seed discs was used to plant maize kernels 5 cm deep on 23 Apr 2008, 22-23 Apr 2009, and 29 Apr 2010. Conventional disk tillage was used in all environments. O-[[2-(1, 1-Dimethylethyl)-5-pyrimidinyl]-O-ethyl O-(1-meth-

Table 1 - Mean squares and level of significance for environment, hybrid, and target population influence on maize grain yield and yield components and lodging.

Source	df	Grain Yield	Ears m^{-2}	Kernel Weight	Bulk Density	Lodging
Environment (E)	4	129.9**	2.76*	540.6**	22683**	1613**
Error A	10	7.7	0.70	19.4	1346	84
Hybrid (H)	3	5.5**	0.38*	35.4**	1073**	189**
E x H	12	1.7*	0.44**	10.4**	169**	101**
Error B	30	0.6	0.10	1.0	30	19
Target Population (P)	5	22.3**	142.97**	248.4**	550**	1065**
E x P	20	2.7**	1.42**	19.9	148**	220**
H x P	15	1.9**	0.25**	2.52	61	21
E x H x P	60	0.8	0.11	1.7	40	18
Residual	200	0.8	0.11	2.0	43	17

* Significant at P ≤ 0.05, ** Significant at P ≤ 0.01

ylethyl) phosphorothioate] (0.164 kg a.i. ha^{-1}) and cyfluthrin [cyano(4-fluoro-3-phenoxyphenyl)-methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-carboxylate] (0.008 kg a.i. ha^{-1}) were applied at planting for CRW control on hybrids without transgenic CRW resistance.

Weeds were controlled with application of recommended pre- and post-emergent herbicides and inter-row cultivation. In irrigated environments, soil was probed to a depth of 90 cm and the «feel method» was used to determine available water content and schedule irrigation applications (Melvin and Yonts, 2009). A single application of 37 mm irrigation water was applied on 25 July 2008. In 2009, three applications of 37 mm irrigation water occurred on 2, 15, and 31 Aug for a total of 111 mm. Presence of gray leaf spot (*Cercospora zeae-maydis* - GLS) influenced maize plant health in 2009 but was not treated.

Maize grain yield, number of ears, and lodging (stalk and root) data were collected from three of the middle rows of each plot on 3 - 10 Oct 2008, 13 - 20 Oct 2009, and 27 Sep - 1 Oct 2010 for the rainfed environments and on 16 - 20 Oct 2008 and 27 Oct - 6 Nov 2009 for the irrigated environments. Plants were considered to be stalk lodged when broken below the ear node and root lodged when leaning at more than a 45° angle (compared to the original upright orientation of the plant). Maize grain yield was determined by harvesting three of the middle rows of each plot with a John Deere (Deere & Company, One John Deere Place, Moline, IL 61265-8098) 3300 combine. A weigh bucket located inside the grain tank equipped with Avery Weigh-Tronix weigh bars (Avery Weigh-Tronix, 1000 Armstrong Drive, Fairmont, MN 56031-1439) was used to determine grain mass. Grain water content was measured for each plot using a Burrows Digital Moisture Computer 700 (Seedburo Equipment Company, 2293 S MT Prospect Road, Des Plaines, IL 60018) and grain mass was adjusted to a constant water concentration of 155 g kg^{-1}. Bulk density was measured with a DICKEY-john GAC 2100 (Dickey-john Corporation, 5200 DICKEY-john Road, Auburn, IL 62615). Kernel weight was determined by counting 100 kernels and weighing with an Ohaus Scout Pro scale (Ohaus Corporation, 7 Campus Drive, Suite 310, Parsippany, NJ 07054).

Data were analyzed using the statistical software R (R Core Team, 2012). Analysis was conducted with environment, hybrid, target population, and their interactions considered fixed effects and replication and interactions with replication considered random effects. Pre-determined single degree-of-freedom contrasts were used for mean separation of discrete variables environment and hybrid and to determine the response shape of the continuous variable target population and interaction effects. Pearson correlations were calculated to identify interrelationships among measured parameters.

Results and Discussion

Seasonal Climatic Conditions

Seasonal rainfall and monthly air temperatures were lowest in 2009 with seasonal rainfall approximately equal to the 52-yr average and air temperatures 1°C lower than average. In 2008 and 2010, seasonal rainfall was much higher than the average. In all years, rainfall was above average during the month of June and in July 2008 and 2010 and in Aug 2009. Monthly average air temperatures were near the 52-yr average in 2008 and approximately 1°C higher in 2010. The Oct average temperature in 2009 was approximately 5°C less than in 2008 and 2010. The cool temperatures in 2009 delayed physiological maturity and in-field drying of grain.

Yield and Yield Components

Maize grain yield was influenced by environment x hybrid (E x H), environment x target population (E x P), and hybrid x target population (H x P) interaction effects (Table 1). Bt hybrids yielded 0.6 Mg ha^{-1} more than non-Bt hybrids with insecticide application at planting in 2008 (Table 2), similar to results of Stanger and Lauer (2006), while no differences were found in 2009 and 2010, which agrees with Coulter et al (2010). No difference in grain yield was found

Table 2 - Environment and hybrid influence on maize grain yield and yield components.

Environment		Grain Yield (Mg ha^{-1})				Ears m^{-2} (no.)				Kernel Weight (g 100-$kernels^{-1}$)			
Year	Water Regime	DKC 58-16	DKC 58-19	DKC 61-69	DKC 61-72	DKC 58-16	DKC 58-19	DKC 61-69	DKC 61-72	DKC 58-16	DKC 58-19	DKC 61-69	DKC 61-72
2008	Rainfed	12.7	12.3	13.0	12.2	7.8	7.7	7.7	7.5	35.9	34.9	37.9	37.5
2008	Irrigated	12.8	12.5	13.4	12.5	7.3	7.1	7.4	7.2	38.5	36.7	40.0	38.2
2009	Rainfed	12.9	12.7	13.3	12.8	7.2	7.4	7.0	7.2	37.8	37.0	37.1	36.5
2009	Irrigated	12.5	12.9	12.4	13.2	7.2	7.3	7.6	7.3	38.1	37.7	38.1	37.7
2010	Rainfed	10.2	9.9	9.6	9.7	7.1	7.3	7.5	7.5	32.0	30.9	31.6	31.6
Mean		12.0	12.0	12.6	12.2	7.3	7.3	7.4	7.3	36.5	38.5	36.9	36.3
Significant Contrasts:		2008 vs 2009 and Bt vs non-Bt ($P = 0.01$)				2008 vs 2009 and Bt vs non-Bt ($P < 0.01$) Rainfed vs Irrigated and Bt vs non-Bt ($P = 0.04$) Rainfed 2008-2009 vs 2010 and DKC 61 Hybrids ($P = 0.01$) Rainfed 2008-2009 vs 2010 and DKC 58 vs DKC 61 Hybrids ($P < 0.01$)				2008 vs 2009 and Bt vs non-Bt ($P < 0.01$) 2008 vs 2009 and DKC 58 vs DKC 61 Hybrids ($P < 0.01$) Rainfed vs Irrigated and DKC 58 vs DKC 58 vs DKC 61 Hybrids ($P = 0.03$)			

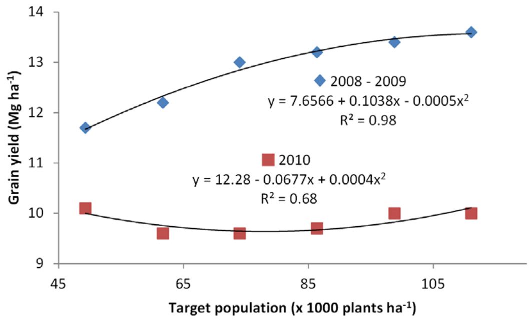

Figure 1 - Year and target population influence on maize grain yield under rainfed conditions (Contrast: 2008-2009 vs 2010 and target population quadratic, $P < 0.01$).

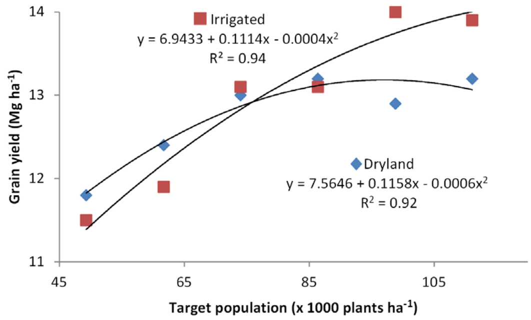

Figure 2 - Water regime and target population influence on maize grain yield (Contrast: Rainfed vs Irrigated and target population quadratic, $P = 0.05$).

between the Dekalb DKC 58 and DKC 61 families of hybrids. Grain yield was 0.3 to 0.4 Mg ha^{-1} greater in 2009 than 2008. Air temperatures in 2009 were lower between the months of June and Oct which delayed maturity and contributed to higher grain yield due to an extended grain fill period (Gambín et al, 2006; Egli, 2011). Likewise, higher temperatures in 2010 between June and Sept led to more rapid maturity and a shorter grain fill period, contributing to lower yield.

Contrast statements for the E x P interaction indicated that maize grain yield increased quadratically with increasing target population under rainfed conditions in 2008 and 2009, while in 2010, maize yield was lower and target population had no influence (Figure 1). Maize grain yield was similar under rainfed and irrigated conditions when target population was below 86400 plants ha^{-1}; however, above this target population maize yield was greater under irrigated conditions (Figure 2).

Grain yield of Dekalb DKC 58-16 and 58-19 increased linearly from 11.0 to 13.1 Mg ha^{-1} [$y = 9.2727 + 0.0363x$ (x 1000 plants ha^{-1}), $R^2 = 0.93$] as target population increased from 49,300 to 111,100 plants ha^{-1} while yield of Dekalb DKC 61-69 and 61-72 responded to target population with a flatter linear increase of 11.8 to 12.7 Mg ha^{-1} [$y = 11.22 + 0.0141x$ (x 1000 plants ha^{-1}), $R^2 = 0.74$]. Dekalb DKC 58-16 and 58-19 produced lower grain yield with low target population and higher grain yield with high target population when compared to Dekalb DKC 61-69 and 61-72. This was likely due to genetic differences between hybrid families such as dry matter production per plant and plant population response (Cox, 1996) as well as the 3-day difference in relative maturity. There was no difference in grain yield response to target population between Bt and non-Bt hybrids.

The number of ears m^{-2} was affected by E x H, E x P, and H x P interaction effects (Table 1). Dekalb DKC 58-16 and 61-69, the Bt hybrids, produced 0.2 more ears m^{-2} than Dekalb DKC 58-19 and 61-72 in the average temperature, high rainfall 2008 year while no differences were found in the cool temperature, average rainfall 2009 and high temperature, high rainfall 2010 years (Table 2). Dekalb DKC 61-69 and 61-72 produced 0.2 more ears m^{-2} in the irrigated environment in 2008 but not in 2009 or 2010. The number of ears m^{-2} increased linearly with target population in 2008 and 2009 as previously reported by Maddonni and Otegui (2004). No difference in ears produced m^{-2} was found between Bt and non-Bt maize hybrids (data not presented).

The target population main effect and E x H interaction effect influenced kernel weight (Table 1). Kernel weight decreased from 39.0 to 34.2 g 100-kernels^{-1} as target population increased from 49300 to 111100 plants ha^{-1}, consistent with results of Maddonni and Otegui (2006). Bt hybrids produced 1.3 g heavier 100-kernel weight in 2008 while no differences were found in 2009 and 2010 (Table 2). Dekalb DKC 61-69 and 61-72 produced 1.9 g heavier 100-kernel weight than Dekalb DKC 58-16 and 58-19 in 2008 but not in 2009 or 2010.

Main and interaction effects on grain bulk density were minimal compared to other yield components. Grain bulk density was 20 to 43 kg m^{-3} lower in 2010 than in 2008 and 2009. Water regime and target population had less effect on bulk density. The E x H interaction effect was declared significant (Table 1); however, differences were small and not influenced greatly by environment or hybrid (data not presented).

Lodging (stalk and root) was affected by the E x H and E x P interaction effects (Table 1). Greater lodging occurred in 2009 (Table 3), likely the result of low temperatures which delayed physiological maturity and presence of GLS which decreased plant health. The Bt maize hybrid Dekalb DKC 58-16 had lower percent lodging than the non-Bt hybrid Dekalb DKC 58-19 in all environments (Table 3), similar to reports of Stanger and Lauer (2006). In contrast, the Bt hybrid Dekalb DKC 61-69 had lower percent lodging than the non-Bt hybrid Dekalb DKC 61-72 in the 2008 rainfed environment and the irrigated environments and higher lodging in the 2009 and 2010 rainfed environments. Percent lodging was lower in the rainfed environment than in the irrigated environment in 2008 but 6 to 18% greater in 2009. Lodging increased linearly as target population increased as previously reported by Pedersen and Lauer (2002) and Stanger and Lauer

Table 3 - Environment and hybrid influence on maize lodging.

Environment		Lodging (%)			
Year	Water Regime	DKC 58-16	DKC 58-19	DKC 61-69	DKC 61-72
2008	Rainfed	2.5	6.4	1.4	5.8
2008	Irrigated	2.9	8.2	6.0	9.3
2009	Rainfed	12.9	15.6	20.2	11.6
2009	Irrigated	2.2	5.1	2.8	5.3
2010	Rainfed	9.3	11.9	10.5	8.1
Mean		5.9	9.4	8.2	8.2
Significant Contrasts:		2008 vs 2009 and Bt vs non-Bt ($P < 0.01$) Rainfed vs Irrigated and Bt vs non-Bt ($P < 0.01$)			

(2006). The rate of increase was greater in 2009 [$y = -10.784 + 0.2533x$ (x 1000 plants ha^{-1}), $R^2 = 0.97$] than 2008 [$y = -1.6757 + 0.0874x$ (x 1000 plants ha^{-1}), $R^2 = 0.92$] and in rainfed [$y = -12.766 + 0.288x$ (x 1000 plants ha^{-1}), $R^2 = 0.90$] rather than irrigated [$y = 0.6371 + 0.0572x$ (x 1000 plants ha^{-1}), $R^2 = 0.90$) environments. Percent lodging was not associated with grain yield; however, it was positively correlated with the number of ears m^{-2} ($r = 0.28$, $P < 0.01$) and negatively correlated with kernel weight ($r = -0.42$, $P < 0.01$) and bulk density ($r = -0.31$, $P < 0.01$).

Conclusion

Bt hybrids yielded slightly more than non-Bt hybrids in environments with no detectable infestation of ECB or CRW based upon visual observations in-season and during harvest; however, Bt hybrids did not respond differently to target population. Increasing target population increased grain yield in rainfed and irrigated environments in years with favorable rainfall distribution and below average air temperatures. Percent lodging increased with increasing target population but response was not consistent across environments or between Bt and non-Bt hybrids. This study found little difference in maize grain yield and percent lodging between Bt and non-Bt hybrids suggesting that seed and insecticide cost, potential for ECB and CRW infestation, and environmental concerns related to soil insecticide usage should be used in determining if Bt hybrids are necessary. Non-Bt hybrids combined with soil insecticide application at planting are a viable alternative if corn rootworm resistance does occur.

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